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EP 0 570 357 B1



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(19) (11) EP 0 570 357 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention of the grant of the patent:
25.05.1997 Bulletin 197/26

(21) Application number: 93890106
(22) Date of filing: 13.05.1993

(23) Priority: 14.05.1992 AT 98792
29.08.1992 US 932787

(51) Int.Cl.: C12N 15/49, C12N 15/62,
A61K 39/21, G01N 33/569,
A61K 39/295, A61K 39/145,
A61K 39/299, C07K 14/16,
C07K 14/02

(54) Peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates
Peptides induisant des anticorps, qui neutralisent des isolats d'HIV-1 qui divergent génétiquement

(64) Designated Contracting States:
BE CH DE DK ES FR GB IE IT LI NL PT SE

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• 8TH INTERNATIONAL CONFERENCE ON AIDS JULY 19-24 1992 vol. 8, no. 2, AMSTERDAM, NL M.PÜRTSCHER ET AL: "Human monoclonal antibodies against HIV-1 in a long-term in vitro neutralization study"; PA73 abstract no. PoA 242*

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Description

This invention refers to peptides that induce antibodies which neutralize genetically divergent HIV-1 isolates. These peptides are applied with an adjuvant, as recombinant fusion proteins, chemically coupled to carrier molecules, as recombinant chimerical viruses or as recombinant antibodies. In addition, the stage of infection can be determined and the progression of the infection can be predicted with these peptides.

INTRODUCTION

10 The acquired immunodeficiency syndrome (AIDS) is the late stage clinical manifestation of long term persistent infection with human immunodeficiency virus type 1 (HIV-1). Immune responses directed against the virus and against virus-infected cells during the persistent infection usually fail to mediate resolution to the infection. A possibility to elicit an immune response that can prevent the establishment of a persistent infection or that can prevent the progression to AIDS are vaccines. Most vaccine strategies against HIV-1 are directed against the surface glycoprotein gp160 which is made up of gp120 and gp41 and is responsible for virus binding to the cellular receptor CD4 and fusion activity.

However, in contrast with gp160 several phenomena that argue against the use of whole gp160 or gp120 as an immunogen were observed. In vitro experiments showed, that synergism between HIV-1 gp120 and gp160-specific antibodies block human T cell activation (1). This result supports the hypothesis, that also *in vivo* that humoral immune response against gp120 of HIV-1 suppresses T-cell activation and might be one reason for immunodeficiency. The proposed mechanism for this phenomenon is cross-linking and modulation of CD4 molecules through gp120 and anti-gp120. Experiments from Kion et al. (2) suggest, that sequence homologies between gp160 and class II MHC molecules lead to immunodeficiency. In addition, a number of antigenic domains on gp160 are known in context with gp160 could be avoided by using synthetic peptides or other subunit vaccines that only contain immunogenic and neutralizing epitopes as immunogens. Immunogenic peptides corresponding to parts of different viral proteins were already used for successful immunization (4,5). The use of synthetic peptides as immunogens offers a number of advantages. The antibodies produced have a predilection for specific regions, and in the case of viruses, they can be selected to represent structures on the surface of viruses. The synthetic polypeptides are also interesting in that they can induce antibody responses not seen under normal conditions.

For example, it was found that in the hemagglutinin of influenza virus there are five major antigenic regions and that under conditions of natural infection the immune response includes antibodies only to those regions. With synthetic polypeptides, an immune response against other regions of the hemagglutinin polypeptide can be generated, and these antibodies have been found to be capable of neutralizing the virus. Therefore it is possible to induce neutralizing antibodies that have a broader reactivity than antibodies induced by whole proteins (4). In addition, immunizations with synthetic polypeptides as are derived from the nucleotide sequence of foot and mouth disease virus (FMDV) are described. In contrast to immunizations with the corresponding whole protein of FMDV, immunizations with these peptides lead to neutralizing antibodies which were also protective (5). Furthermore, a peptide containing part of the V3 loop of gp120 from HIV-1 isolate IIIB was shown to induce a protective immune response against a virus challenge with the same HIV-1 isolate (7,8).

Because synthetic peptides themselves have poor immunogenicity, they have to be coupled to molecules that provide an adjuvant effect such as proteins, toxoid or keyhole limpet hemocyanin (5). Another possibility is to clone small peptides as fusion peptides with glutathione-S-transferase of Schistosoma japonicum (9,10). In addition, attenuated viruses such as vaccinia, pox, Sabín type 1 or influenza NAE-NIS can be used as vectors for immunogens. Vaccinia virus is used frequently as a vector of foreign genes of multiple pathogens. For example, rabbits inoculated with recombinant vaccinia virus containing sequences from hepatitis B surface antigen (HBsAg), herpes simplex virus glycoprotein D, and influenza virus hemagglutinin produced antibodies to all three foreign antigens (11). Furthermore, a chimerical pox virus that expressed an epitope from gp41 of HIV-1 induced neutralizing antibodies against gp41 in rabbits (12). Since recently it is also possible to change the genome of influenza virus by *in vitro* mutagenesis (13). By means of this technique, it was possible to engineer a stable attenuated influenza A virus (14). In addition, by using this technique it was also possible to construct an inactivated chimerical virus, in which a six-amino-acid loop contained in the antigenic site B of the hemagglutinin of an H1 subtype was replaced by its corresponding structures of subtypes H2 and H3 (15). An advantage of influenza virus in this context is the availability of many variants so that repeated vaccination may be possible. Furthermore, influenza virus induces strong sialoglycan and cellular immune responses, which may be advantageous for an anti-HIV-1 vaccine approach. In addition, it is unlikely that influenza virus is associated with the development of malignancies. There is no DNA phase involved in the replication of influenza viruses, which excludes the possibility of chromosomal integration of viral influenza genes.

The use of antibody-like peptides is another possibility to achieve a specific immune reaction. Antibody-like peptides are antibodies that specifically recognize and bind the antigen binding site of another antibody. As the combining sites of antibodies can be structurally looked at as a mirror image of the epitope that is bound, an antibody-like antibody

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corresponds to the mirror image of this primary mirror image, which means that an antiidiotypic antibody displays the internal image of the epitope that is bound by the idiotypic antibody. Although one cannot always expect to find complete identity between the structure of the amino acid sequence respectively of the antiidiotypic antibody with that of the epitope, one can however see effects in practice that allow the conclusion that there is a structural, sequential or functional similarity between an antiidiotypic antibodies and the respective epitopes. The use of antiidiotypic antibodies as a vaccine was initially proposed by Nisonoff and Lampert (15). In the case of African Sleeping Disease, it was first shown that a protective immune response against the causative agents, *Trypanosoma brucei* merozoites, could be elicited in BALB/C mice by vaccinating the mice with an antiidiotypic antibody (17). In the case of viral antigens, the formation of antiidiotypic antibodies to a neutralizing epitope on the hemagglutinin molecule of *Picornavirus Type III* was investigated. These antiidiotypic antibodies recognized the cellular receptor of *Flavivirus-Hemagglutinin* on both, cytotoxic T-cells and neuronal cells, and were able to induce in mice a humoral as well as a cellular immune response specific to *Flavivirus-Hemagglutinin* (18, 19, 20).

DETAILED DESCRIPTION OF THE INVENTION

Peptides comprising 6 amino acid residues (aa) that bind specifically to the monoclonal antibody 2F5 were used as immunogens to induce neutralizing antibodies against HIV-1. For identification of these peptides overlapping fragments of gp41 (HIV-1 isolate BH-10) were cloned as fusion peptides with glutathione transferase. The different fusion peptides were obtained through hyperylation of gp41 corresponding oligonucleotides which were cloned between the Bam HI and the Eco RI site of the plasmid pGEX-2T (Pharmacia). The recombinant plasmids were transformed into the *E. coli* strain DH5α and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). The *E. coli* extract was then purified with glutathione-Sepharose 4B columns, eluted on sodiumdodecylsulfate-polyacrylamide gels, separated by electrophoresis and protein expression was analyzed by silver staining. Fusion peptides that were reactive with the monoclonal antibody 2F5 were identified by immunoblotting. Using this method peptides which bind to the monoclonal antibody 2F5 were identified. Figure 1 shows Western blots of fusion peptides with overlapping fragments of gp160 of HIV-1 (isolate BH-10), in contrast to constructs that comprise aa 597 to 617, 634 to 677 and 648 aa within the sequence from position 648 to 657 (gp160). Based on these results, overlapping 6-mer peptides of this region were used with the glutathione S-transferase. As shown in figure 2, the peptide containing the amino acid sequence GLU1 LEU1 ASP1 LYS1 TRP1 ALA1 (aa 652-657) was highly reactive with the antibody 2F5 whereas for peptides containing the amino acid sequence LEU1 ASP1 TRP1 ALA1 SER1 LEU1 (aa 664-669) or ASP1 LYS1 TRP1 ALA1 (aa 663-668) no reactivity with the monoclonal antibody was significantly lower. A peptide containing amino acid sequence LEU1 GLU1 ASP1 LYS1 TRP1 ALA1 (aa 651-656) showed no reactivity at all. These data suggest that the epitope of the monocolonal antibody comprises the amino acid sequence GLU1 LEU1 ASP1 LYS1 TRP1 ALA1 that correspond to aa 652-657. In this context both, a synthetic peptide corresponding to this epitope sequence and a fusion protein containing this sequence were able to inhibit neutralization mediated by the 2F5 antibody (Fig. 4).

Sequence comparison of the region revealed that the corresponding amino acid sequence is highly conserved between otherwise genetically highly divergent HIV-1 isolates (Table 2a). We also were able to show that fusion peptides with amino acid substitutions according to different HIV-1 isolates- in this region were also reactive with the 2F5 antibody (Fig. 1c).

The presence of antigenic domains around this region has been reported previously (21-22). Townsend et al. reported of a monoclonal antibody, that reacted with a peptide corresponding to aa 643 to 652 of gp160. In addition, Bodenham et al. reported that HIV-1 antibody-positive human sera were reactive with a peptide corresponding to region 657-671. However, in both cases a specific epitope was not identified. The monoclonal antibody reported by Townsend et al. had no neutralizing activity. Also the sera reactive with the peptide 657-671 of Bodenham et al. showed just partial neutralizing activity in different neutralization assays this group was able to show neutralizing activity against HIV-1 isolate III-B but not against SF2 and RF.

In contrast to this result, the monoclonal antibody 2F5 neutralizes a variety of different HIV-1 isolates including SF2 and RF (table 1). These data suggest that the antibodies of the sera reported by Bodenham et al. as well as the monoclonal antibody (reported by Townsend et al.) have a different specificity and recognize a different epitope than the antibody 2F5.

The application of the peptides described in the present invention as immunogen has several advantages. They comprise just 6 aa. Thus, other gp160 peptide sequences which include antibodies that enhance HIV-1 infection or lead to immunosuppression can be avoided (23). Furthermore an effective HIV-1 vaccine should induce an immune response against HIV-1 isolates that vary considerably in their genomic sequences. In this context sequence comparison in the region of the gp25 epitope revealed that the epitope of the 2F5 antibody is highly conserved between different

isolates (Table 2a). Since peptides with aa substitutions corresponding to genetically different HIV-1 isolates were reactive with the 2F5 antibody (Fig. 1c), it is likely that antibodies induced by peptides described in the present invention are directed against a variety of divergent HIV-1 isolates. In addition, the 2F5 antibody showed neutralizing activity against a wide variety of genetically different HIV-1 isolates which proves that peptides described in the present invention are presented as neutralizing epitope (Table 1).

In order to know which variations of the epitope sequence are binding to the monoclonal antibody 2F5 we undertook a peptide mapping with a random hexapeptide library displayed on protein III of a filamentous phage (22a). The hexapeptide sequences of the studied phage particles were compared (Table 2b).

There is a wide range of variation in the progression of HIV-1 related disease in different HIV-1 infected persons. In many cases HIV-1 infection ends up in AIDS-related complex (ARC) and AIDS within some years, while some HIV-1 positive persons remain asymptomatic. It has been shown that antibody-titers against certain peptide epitopes are much lower in AIDS-patients compared to asymptomatic status (23). We found a significant correlation between the antibody-titers to the peptides described in the present invention and HIV-1 related disease progression (Fig. 2). Patients number 20, 25, 29, 51, 44 and 46 who have a high antibody-titer to peptides described in this invention (Fig. 2), did not show any progression in disease within the last five years so far. This means that generation of antibodies induced by peptides described in the present invention can inhibit or at least reduce the progression of HIV-1 related disease. The fact that there are rarely high antibody-titers to peptides described in this invention found in sera of HIV-1 positive patients indicates that those epitopes on gp160 are not recognized easily by the human immune system, resulting in low HIV-1 neutralizing antibody titers specific to these epitopes. An objective of the present invention is also to present the peptides described in the invention in a proper form and to induce a sufficient neutralizing immune response.

Example 1:

Cloning and expression of peptides described in the invention as fusion proteins with glutathione S-transferase (GST) and immunizations of mice with these peptides is described. All cloning methods were done according to standard procedures (24). Oligonucleotides corresponding to the peptides described in the invention were hybridized and cloned between the Bam HI and Eco RI site of the plasmid pGEX-2T (Pharmacia). By this the NH₂-terminal ends of these peptides were fused with the COOH-terminal ends of the GST. In addition, a stop codon was added to the COOH-terminal ends of the gp41 peptide sequences. These constructs were transformed into *E. coli* DH5α and expression of the fusion proteins was induced with isopropylthiogalactoside (IPTG). After three hours of induction bacteria were harvested by centrifugation, suspended in phosphate buffered saline (PBS, pH 7.2) containing 1% Triton-X-100 and sonicated. Bacterial debris were spun down by centrifugation and the supernatant was loaded on glutathione-Sepharose 4B columns (Pharmacia). Elution of the fusion proteins was done with 20 mM glutathione and 120 mM NaCl in 10 mM Tris-HCl (pH 8.0). Purified fusion proteins obtained with this procedure were used for immunizing mice according to standard procedures. As a control, mice were immunized with GST prepared in the same way as the fusion proteins. Sera from mice taken one week after the last immunization showed high neutralizing titers against peptides described in the invention and inhibited HIV-1 replication *in vitro* (Fig. 3b and 3c).

Example 2:

Example 2 describes the expression of peptides sequences described in the invention as part of the hemagglutinin of influenza A virus. *In vitro* mutagenesis was used to introduce this peptide sequence into the antigenic sites A,B,C, D and E of the hemagglutinin of influenza A virus (26, 27). These chimerical DNA-constructs were then "FNP-transfected" into influenza H/M/WSN virus (13). These chimerical influenza/HIV viruses had the antigenic properties of said peptide. In antibody-adsorption experiments these chimerical viruses inhibited HIV-1 neutralization through the antibody 2F5 (Fig. 3b). Antisera of mice immunized with the chimerical viruses were reactive with said peptides (Fig. 3c). Furthermore, *in vitro* these antisera neutralized different HIV-1 isolates (Fig. 3c).

Example 3:

Example 3 describes the expression of peptides described in the invention as part of a so called "immunological supermolecule", where the peptide sequence is inserted into the linker which connects the variable regions of the heavy and light chain of an immunoglobulin molecule. Specifically a single chain Fv construct of a neutralizing antibody 2F5 was made according to standard procedures (27). In this construct peptide sequences described in the invention were inserted into the linker which connects the variable regions of the light chain with the variable region of the heavy chain. This recombinant protein was expressed in *E. coli* and purified according to standard procedures. Two functions were observed with this construct. First this construct showed the antigen binding properties

of the original antibody and in addition, this construct induced, when injected into mice, antibodies that neutralized different HIV-1 isolates (Fig. 3c). This "immunological supermolecule" provides the possibility to obtain an active and passive immunization at the same time. Basically, in such a construct the antigen-binding neutralizing properties of an antibody and the presentation of a neutralizing epitope are combined. In already HIV-1 infected persons the progression of infection could be slowed down with the first application by the antigen binding neutralizing properties, before the decisive onset of the immune system is triggered by the neutralizing epitopes of this molecule. Thus the usual observed time lag between immunization and effective immune response of a typical active immunization could be overcome. In addition, it is most likely that during neutralization of already present HIV-1 virions the presentation of the epitope is very efficient.

Example 4:

Example 4 describes the formation of antidiotypic antibodies to antibody 2F5 as well as the production of an antidiotypic antibody by means of *in vitro* recombinant techniques.

Antibody 2F5 was used to immunize mice in order to induce the formation of antidiotypic antibody. The immunization scheme used was according to standard procedures in order to enhance the frequency at which antidiotypic antibodies are developed in the animal. The polyclonal sera such obtained were tested for their immunoreactivity, whereby it was determined by means of antigen-competition ELISA that a part of the humoral immune response was indeed directed against the combining site of the antibody 2F5. Thus it was proven that in those sera antidiotypic antibodies were present. In order to test the concept of vaccination by means of antidiotypic antibodies, the sera containing antidiotypic antibodies were subsequently used to immunize another group of mice. After completion of this immunization procedure, it was possible to detect an immune response to the antidiotypic sera that was quantitatively comparable to the above described immune reaction against the HIV-1 peptide-part of the glutathione-S-transferrase fusion protein described in example 1.

Since it was now proven, that the described peptide has the qualities necessary to act as an immunogen, and since furthermore it had been shown that by using antidiotypic antibodies, an antidiotypic antibody was constructed by means of *in vitro* recombination techniques in order to achieve this goal, one or more hypervariable regions (or parts thereof) of an existing, molecularly cloned antibody were substituted by peptide sequences described in the invention. The respective constructs, where expressed as single chain Fv fragments in E. coli, and the recombinant proteins where purified according to standard methods, immunization of mice with the antidiotypic proteins such produced lead to a HIV-1 neutralizing immune response (Fig. 3c).

Example 5:

Example 5 describes the peptide mapping with a random hexapeptide library and immunizations of mice with phage containing peptides according to SEQ ID NO: 10 through SEQ ID NO: 25. The monoclonal antibody 2F5 was coated onto polystyrene tubes (Maxisorp, Nunc, Denmark) at a concentration of 5 µg/ml in coating buffer (0.1 M Na-carbonate buffer, pH 9.5) overnight at 4°C. After washing with PBS, the surface was blocked with PBS containing 5% w/v skimmed milk powder at 35°C for 2 hours. Washing with PBS was followed by incubation of a hexapeptide phage display library (10^{11} transciption units in TBS/PBS including 0.5% w/v Tween 20) overnight at 5°C. Extensive washing with TBS was followed by elution of phage with elution buffer (0.1 N HCl/Glycine pH 2.2, 1 mg BSA/ml). The eluate is neutralized with 1 M Tris and used for infection of E. coli K91 Kan. Phage is prepared from the infected culture by the methods described (22a). The procedure is repeated 3 times. The final eluate was used to produce transduced E. coli K91 Kan. DNA of these clones was sequenced and the respective phage displayed hexapeptide sequence was derived by computer translation.

Clones with the SEQ ID NO: 10 through 25 (Table 2) were used for phage preparation and the respective phages were injected into mice. After two booster injections the respective sera were tested for HIV-1 neutralizing activity. All of these sera were neutralizing in vitro. The control serum was produced by immunization with wild-type phage 11 and did not neutralize HIV-1. In addition, oligonucleotides coding for SEQ ID NO: 10 through 25 were introduced into gene VIII of ts₄₄ between aa 27 and 28 of the immature protein VIII by standard cloning techniques. The recombinant phages were produced in E. coli K91 Kan, purified by standard techniques (PEG mediated precipitation followed by CsCl gradient centrifugation) and used as immunogen. The respective sera were found to be neutralizing in HIV-1 neutralizing assays whereas anti-wild type ts₄₄ was not.

Sequence Listing:

SEQ ID NO: 1

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate BH10 POSITION OF THE SEQUENCE IN GP160: from residue 682 to 687

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: Translated from GenBank accession M15654, nucleotides 7583 to 7580

10

Glu Leu Asp Lys Trp Ala
1 5 6

15

SEQ ID NO: 2

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate J54/28 POSITION OF THE SEQUENCE IN GP160: from residue 655 to 660

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: Translated from GenBank accession M37576, nucleotides 1983 to 1980

20

Glu Leu Asn Lys Trp Ala
1 5 6

25

SEQ ID NO: 3

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate patient 3U POSITION OF THE SEQUENCE IN GP160: from residue 184 to 189

PROPERTIES: Epitope of a human monoclonal antibody directed against HIV-1 GP160

REFERENCE: Translated from GenBank accession X61332, nucleotides 490 to 507

30

Glu Leu Asp Lys Trp Asp
1 5 6

35

SEQ ID NO: 4

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: GP160 of HIV-1 isolate SF170 POSITION OF THE SEQUENCE IN GP160: from residue 687 to 692

LENGTH OF SEQUENCE: 6 amino acid residues
 TYPE OF SEQUENCE: amino acid sequence
 TYPE OF FRAGMENT: inner fragment

5 ORIGIN: p3 fusion protein of filamentous phage I USES POSITION OF THE SEQUENCE IN p3; from residue
 4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
 SEQ ID NO: 12

10 GLY Arg Asp Lys Trp Ala
 1 5 6

15 SEQ ID NO: 13

LENGTH OF SEQUENCE: 6 amino acid residues
 TYPE OF SEQUENCE: amino acid sequence
 TYPE OF FRAGMENT: inner fragment

20 ORIGIN: p3 fusion protein of filamentous phage I USES POSITION OF THE SEQUENCE IN p3; from residue
 4 to 9

25 PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
 SEQ ID NO: 14

30 GLY Ala Asp Lys Trp Ala
 1 5 6

35 SEQ ID NO: 13

LENGTH OF SEQUENCE: 6 amino acid residues
 TYPE OF SEQUENCE: amino acid sequence
 TYPE OF FRAGMENT: inner fragment

40 ORIGIN: p3 fusion protein of filamentous phage I USES POSITION OF THE SEQUENCE IN p3; from residue
 4 to 9

45 PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
 SEQ ID NO: 14

50 GLY Ala Asp Lys Trp Ala
 1 5 6

55 SEQ ID NO: 14

LENGTH OF SEQUENCE: 6 amino acid residues
 TYPE OF SEQUENCE: amino acid sequence
 TYPE OF FRAGMENT: inner fragment

59 ORIGIN: p3 fusion protein of filamentous phage I USES POSITION OF THE SEQUENCE IN p3; from residue
 4 to 9

64 PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
 SEQ ID NO: 18

69 LENGTH OF SEQUENCE: 6 amino acid residues
 70 TYPE OF SEQUENCE: amino acid sequence
 71 TYPE OF FRAGMENT: inner fragment

75 ORIGIN: p3 fusion protein of filamentous phage I USES POSITION OF THE SEQUENCE IN p3; from residue
 4 to 9

80 PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
 SEQ ID NO: 18

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TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 19

Gly Ala Asp Lys Trp Val
1 5 6

SEQ ID NO: 20

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 21

Gly Ala Asp Lys Trp His
1 5 6

SEQ ID NO: 22

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 23

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

Ala Phe Asp Lys Trp Val
1 5 6

SEQ ID NO: 24

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 25

Gly Ala Asp Lys Cys Gln
1 5 6

SEQ ID NO: 26

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 27

Gly Pro Asp Lys Trp Gly
1 5 6

SEQ ID NO: 28

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 29

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 30

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 31

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 32

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 33

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 34

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 35

LENGTH OF SEQUENCE: 6 amino acid residues
TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

ORIGIN: p3 fusion protein of filamentous phage USES POSITION OF THE SEQUENCE IN p3: from residue

4 to 9

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5

SEQ ID NO: 36

LENGTH OF SEQUENCE: 6 amino acid residues

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TYPE OF SEQUENCE: amino acid sequence
TYPE OF FRAGMENT: inner fragment

C₁O₁G₁N₁: p3 fusion protein of filamentous phage f1 USE5 POSITION OF THE SEQUENCE IN p3: from residue

PROPERTIES: hexapeptide binding to monoclonal antibody 2F5
4 to 9
5
ORIGIN: gp160 of HIV-1 isolate (patient 3L)
REFERENCE: GenBank accession X61352
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 490-507

Ala Arg Asp Lys Trp Ala

SEQ ID NO: 26
LENGTH OF SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: Inner fragment

ORIGIN: GP160 of HIV-1 isolate BH10
REFERENCE: GenBank accession M15654
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 7564-7580

gaatttagata aatgggca

30
18
11
1

SEQ ID NO: 27

LENGTH of SEQUENCE: 18 base pairs

TYPE OF SEQUENCE: nucleotide sequence

TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear

TYPE OF SEQUENCED MOLECULE: CUNA10 viral RNA
TYPE OF FRAGMENT: Inner fragment

ORIGIN: gp160 of HIV-1 isolate J54420
REFERENCE: GenBank accession M37576
LOCATION OF THE SEQUENCE IN DATABASE ENTRY: 1963-1980

POSITION OF THE SECRETARIAL IN SERVICE.....

gaatttgaata agtggccaa

59

SEQ ID NO: 28

LENGTH OF SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

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14

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LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

5
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate Z-84
REFERENCE: GenBank accession J03853

10
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2037-2054

caattggaca aatgggca

15
18 15
1 11

SEQ ID NO: 32

20
LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

25
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate CAM1 proviral genome
REFERENCE: GenBank accession D1012
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 8209-8226

30
gatattggata cgtagggca

18 15
1 11

SEQ ID NO: 33

40
LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

45
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate US4/6
REFERENCE: GenBank accession M37491

50
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 2416-2433

gatattggata cgtagggca

18 15
1 11

SEQ ID NO: 34

EP 0 570 357 B1

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

5
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: cDNA to viral RNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: GP160 of HIV-1 isolate S8B
REFERENCE: GenBank accession M77229

10
POSITION OF THE SEQUENCE IN DATABASE ENTRY: 1239-1255

aatgttagatg agtggggca

15
18 15
1 11

SEQ ID NO: 35

20
LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

25
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

30
POSITION OF THE SEQUENCE IN fUSE: 10 to 27

tcgttttgata aytggggcc

35
18 15
1 11

SEQ ID NO: 36

40
LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

45
TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
TYPE OF FRAGMENT: inner fragment
ORIGIN: gene coding for p3 fusion protein of filamentous phage fUSE5

50
POSITION OF THE SEQUENCE IN fUSE: 10 to 27

gggcgtgata agtggggcc

18 15
1 11

SEQ ID NO: 34

POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata aatgggt

18
1 11

5 POSITION OF THE SEQUENCE IN p3: 10 to 27

10 SEQ ID NO: 43

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

15 TYPE OF STRAND: single strand
TOPOLOGY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
ORIGIN: gene coding for p3 fusion protein of filamentous phage lUSE5

20 POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata aatgggtt

18
1 11

25 POSITION OF THE SEQUENCE IN p3: 10 to 27

10 SEQ ID NO: 44

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

30 TYPE OF STRAND: single strand
TOPOMETRY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
ORIGIN: gene coding for p3 fusion protein of filamentous phage lUSE5

35 SEQ ID NO: 45

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

40 TYPE OF STRAND: single strand
TOPOMETRY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
ORIGIN: gene coding for p3 fusion protein of filamentous phage lUSE5

45 POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata aatgtcat

18
1 11

50 POSITION OF THE SEQUENCE IN p3: 10 to 27

ggcgctgata agtggcat

18
1 11

55 SEQ ID NO: 46

LENGTH of SEQUENCE: 18 base pairs
TYPE OF SEQUENCE: nucleotide sequence

60 TYPE OF STRAND: single strand
TOPOMETRY OF SEQUENCE: linear
TYPE OF SEQUENCED MOLECULE: phage DNA
ORIGIN: gene coding for p3 fusion protein of filamentous phage lUSE5

- TYPE OF STRAND: single strand
 TOPOLOGY OF SEQUENCE: linear
 TYPE OF SEQUENCED MOLECULE: phage DNA
 TYPE OF FRAGMENT: linear fragment
 ORIGIN: gene coding for p3 fusion protein of filamentous phage I USES
 POSITION OF THE SEQUENCE IN p3: 10 to 27
- 5
- 10 gcttttgata atgtgggtt
 18
 1 11
- 15 SEQ ID NO: 49
 LENGTH of SEQUENCE: 18 base pairs
 TYPE OF SEQUENCE: nucleotide sequence
 20 TYPE OF STRAND: single strand
 TOPOLOGY OF SEQUENCE: linear
 TYPE OF SEQUENCED MOLECULE: phage DNA
 TYPE OF FRAGMENT: linear fragment
 25 ORIGIN: gene coding for p3 fusion protein of filamentous phage I USES
 POSITION OF THE SEQUENCE IN p3: 10 to 27
- 20
- 20 ggcgcgtgata aatgggtt
 18
 1 11
- 25 SEQ ID NO: 50
 LENGTH of SEQUENCE: 18 base pairs
 TYPE OF SEQUENCE: nucleotide sequence
 30 TYPE OF STRAND: single stand
 TOPOLOGY OF SEQUENCE: linear
 TYPE OF SEQUENCED MOLECULE: phage DNA
 TYPE OF FRAGMENT: linear fragment
 35 ORIGIN: gene coding for p3 fusion protein of filamentous phage I USES
 POSITION OF THE SEQUENCE IN p3: 10 to 27
- 30
- 30 ggcgcgtgata aatgggtt
 18
 1 11
- 35 SEQ ID NO: 50
 LENGTH of SEQUENCE: 18 base pairs
 TYPE OF SEQUENCE: nucleotide sequence
 40 TYPE OF STRAND: single stand
 TOPOLOGY OF SEQUENCE: linear
 TYPE OF SEQUENCED MOLECULE: phage DNA
 TYPE OF FRAGMENT: linear fragment
 45 ORIGIN: gene coding for p3 fusion protein of filamentous phage I USES
 POSITION OF THE SEQUENCE IN p3: 10 to 27
- 40
- 40 gtcgtgtgtata agtggggc
 18
 1 11
- 45
- 45 Literature
- 50
- 50
- 55
- 55
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- 40 Tables

Table 1:

Neutralizing properties of human monoclonal antibody 2F5

a) *in vitro* neutralization assays:

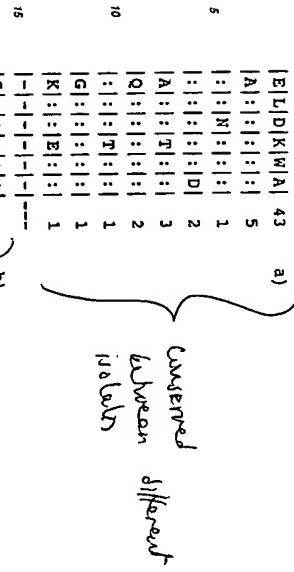
Isolate						
	IIB	MN	RF	SF2	A	C
number of positive tests neutralizing concentration (pg/ml)	8/8	2/2	2/2	n.t.	8/8	4/4

- 45 b) syncitia inhibition assay:
Isolate

	IIB	MN	RF	SF2	A	C
number of positive tests EC ₅₀ (pg/ml)	18/18	11/11	6/10	1/1	1/1	2/3

- 50 a) *In vitro* neutralization assay: Different concentrations of the 2F5 antibody were incubated with culture virus preparations (10^3 - 10^5 TCID₅₀/1 h at 37°C). HEK 293 cells were added to VeroA2 monolayers and incubated for an additional hour at 37°C. After 20 days p24 antigen concentration as indicator for virus replication was determined from supernatants according to standard procedures.

- b) Syncitia inhibition assay: Antibody/tissue mixtures were prepared as described in table 4. To this mixture 10⁵ A2 cells were added and incubated at 37°C. After 5 days syncitia formation as indicator for HIV-1 replication was evaluated. Abbreviations A and C are clinical isolates from Vienna



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Table 2: Peptide sequences bound by antibody 2F5:

a: sequences present on gp160 of different HIV-1 isolates. The number on the right side of each sequence indicates the number of incidences in the databases that were screened (SwissProt and GenPept).

b: binding sequences found by screening a random hexapeptide library expressed on the surface of filamentous phage (sequences already described in a) are not included).

a) *In vitro* neutralization assay: Different concentrations of the 2F5 antibody were added to VeroA2 monolayers and incubated for an additional hour at 37°C. After 20 days p24 antigen concentration as indicator for virus replication was determined from supernatants according to standard procedures.

b) Syncitia inhibition assay: Antibody/tissue mixtures were prepared as described in table 4. To this mixture 10⁵ A2 cells were added and incubated at 37°C. After 5 days syncitia formation as indicator for HIV-1 replication was evaluated. Abbreviations A and C are clinical isolates from Vienna

Figure legends:

isically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.

- Fig.1:** Western blots of fusion peptides. Recombinant protein expressed in *E. coli* were purified as described in example 1 and 100ng of each fusion peptide was fractionated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 20% polyacrylamide gel and electroblotted onto a nitrocellulose filter. The blots were blocked with 0.1% nonfat dried milk in phosphate-buffered saline containing 0.1% Tween for 1 h at room temperature. After washing, blots were incubated with antibody 2F5 (500ng/ml) for 1 h at room temperature. After washing, blots were incubated for 1 h at room temperature with anti-human IgG-alkaline-phosphatase-conjugated. Blots were developed with 1M diethanolamine buffer (pH 9.5) containing 350ug/ml nitro-blue tetrazolium chloride and 350 ug/ml 5-bromo-4-chloro-3-indolyl-phosphate.
- Fig.1a:** Lane 1, glutathione S-transferase (GST); lane 2, amino acids (aa) 597-677 of gp160 fused with GST; lane 3, aa 652-677 fused with GST; lane 4, aa 646-677 fused with GST; lane 5 aa 657-677 fused with GST.
- Fig.1b:** Lane 1, GST; lane 2, GST fused with aa GLULEU ASP LYS TRP ALA (aa 652-657); lane 3, GST with aa LEU ASP LYS TRP ALA (aa 653-658); lane 4, GST with ASP LYS TRP ALA SER LEU (aa 654-659); lane 5, GST with aa LEU GLU LEU ASP LYS TRP (aa 651-656).
- Fig.1c:** Fusion peptides with amino acid substitutions according to HIV-1 isolates with differences in the region of the 2F5 epitope. Amino acid differences are underlined. Lane 1, GST; lane 2, GLU LEU ASP LYS TRP ALA; lane 3, GLU LEU ASP LYS TRP ALA; lane 4, GLY LEU ASP LYS TRP ALA; lane 5, ALA LEU ASP LYS TRP ALA; lane 6, GLULLEU ASN LYS TRP ALA (reaction of this fusion peptide with the 2F5 antibody is not visible in this Western blot, however in ELISA competition assays this peptide was competitive to recombinant gp41 for binding to the 2F5 antibody); lane 7, GLU LEU ASP TRP TRP ALA; lane 8, GLU LEU ASP LYS TRP ASG.

- 10** 10. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.
- 11** 11. Fusion peptides according to claim 10, characterised in that they are part of a virus.
- 12** 12. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.
- 13** 13. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.
- 14** 14. Use of peptides as defined in anyone of claims 1 through 5 to select antibodies or antibody fragments binding to HIV-1 *in vitro*.
- 15** 15. Use of peptides as defined in anyone of claims 1 through 5 in an immunological test *in vitro* to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection, or to make a prognosis on the further progress of infection.
- 16** 16. Use of peptides as defined in anyone of claims 1 to 5 for the manufacture of antidiagnostic antibodies.
- 17** 17. A vaccine against HIV-1 characterised in that it comprises at least one peptide and/or fusion peptide as defined in anyone of claims 1 to 13 and/or at least one antidiagnostic antibody obtained according to claim 16.

Claims for the following Contracting States : BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE

- 1** 1. Peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, characterised in that said peptides are composed according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25.
- 2** 2. Peptides according to claim 1, characterised in that they are genetically encoded by the nucleotide sequence according to anyone of the SEQ ID NO: 26 through SEQ ID NO: 50 or by sequences that are deduced from SEQ ID NO: 26 through SEQ ID NO: 50 by degeneration.
- 3** 3. Peptides according to claim 1 or 2, which upon injection into a mammal either alone or in combination with an adjuvant cause an immune response that leads to the generation of HIV-1 neutralizing antibodies.
- 4** 4. Peptides according to anyone of claims 1 to 3 in combination with an adjuvant, wherein the adjuvant is a substance to which said peptides are bound through chemical interaction.
- 5** 5. Peptides according to claim 4 in the form of fusion peptides, characterised in that a protein or part of a protein is used as the adjuvant, to which said peptides are bound by fusion of the respective nucleotide sequences and subsequent expression of the fusion genes in a biological expression system.
- 6** 6. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 are used as linker or as part thereof in order to link the variable domains of a single chain Fv fragment.
- 7** 7. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a monoclonal antibody.
- 8** 8. Fusion peptides according to claims 5 and 7, characterised in that one or more peptides according to one of the SEQ ID NO: 1 through SEQ ID NO: 25 are expressed as part of one or more hypervariable regions of a monoclonal antibody.
- 9** 9. Fusion peptides according to anyone of claims 5, 7 or 8, characterised in that they are either expressed, or chem-

ically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.

- 10** 10. Fusion peptides according to claim 5, characterised in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.
- 11** 11. Fusion peptides according to claim 10, characterised in that they are part of a virus.
- 12** 12. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.
- 13** 13. Fusion peptides according to claim 10 or 11, characterised in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.
- 14** 14. Use of peptides as defined in anyone of claims 1 through 5 to select antibodies or antibody fragments binding to HIV-1 *in vitro*.
- 15** 15. Use of peptides as defined in anyone of claims 1 through 5 in an immunological test *in vitro* to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection, or to make a prognosis on the further progress of infection.
- 16** 16. Use of peptides as defined in anyone of claims 1 to 5 for the manufacture of antidiagnostic antibodies.
- 17** 17. A vaccine against HIV-1 characterised in that it comprises at least one peptide and/or fusion peptide as defined in anyone of claims 1 to 13 and/or at least one antidiagnostic antibody obtained according to claim 16.

Claims for the following Contracting State : ES

- 1** 1. A method for the manufacture of peptides which bind to antibodies, that show neutralizing activity against genetically divergent strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that oligonucleotides corresponding to one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 are cloned, transformed into and expressed in *E. coli*, preferably *E. coli* DH5 α .
- 2** 2. A method according to Claim 1 characterized in that the peptides are expressed as fusion proteins with glutathione S-transferase (GST), preferably with said oligonucleotides being hybridized and cloned between the Bam HI and Eco RI site of the plasmid pETEX-2T (Pharmacia).
- 3** 3. A method according to Claim 1 or 2, characterized in that after expression of said amino acid sequences and/or said peptides in *E. coli* cells are disrupted and said amino acid sequences and/or said peptides are separated from the liquid fraction and purified.
- 4** 4. A method according to Claim 3, characterized in that disruption of the *E. coli* cells is achieved by sonication.
- 5** 5. A method according to Claim 3 or 4, characterized in that the separation and purification of the amino acid sequences and/or peptides is carried out by affinity chromatography, preferably using a glutathione sepharose 4B column, wherein the amino acid sequences and/or peptides are eluted, preferably with a solution containing glutathione, NaCl and a buffer.
- 6** 6. A method according to Claim 5, characterized in that the elution medium is a solution composed of 20 mM glutathione and 120 mM NaCl in 100 mM Tris-HCl, pH 8.0.
- 7** 7. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that
- 55** a) at least one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 is introduced into antigenic sites of the hemagglutinin of influenza A virus by *in vitro* mutagenesis, thereby leading to chimerical

- DNA constructs,
b) said chimerical DNA constructs thereafter being RNIP-transfected into influenza HK/WSN virus,
- whereby chimerical influenza/HIV viruses are created which exhibit antigenic properties of said fusion proteins, and chimerical influenza/HIV viruses preferably being capable of inducing a neutralizing immune response against genetically divergent HIV-1 strains.
8. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that
- 10 a) a single chain Fv construct of a neutralizing anti-HIV-1p20 antibody is prepared,
b) at least one of the amino acid sequences of SEQ ID NO: 1 through SEQ ID NO: 25 is inserted into the linker which connects the variable regions of the heavy and light chain of an immunoglobulin molecule,
c) said fusion proteins are then expressed as part of said immunoglobulin molecule containing the inserted amino acid sequence.
9. A method for the manufacture of fusion proteins which bind to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and which inhibit the fusion of cells caused by HIV-1, characterized in that one or more hypervariable regions or parts thereof of a monoclonal antibody are substituted by at least one in that one or more hypervariable regions or parts thereof of a monoclonal antibody are substituted by at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25, by using standard methods.
- 10 10. A method according to Claim 9, characterized in that said fusion proteins are expressed as single chain Fv fragments in *E. coli*, thereafter purified and injected into mice, which thereupon leads to the formation of antidiotypic antibodies that are capable of inducing a neutralizing immune response against genetically divergent HIV-1 strains..
- 11 A method for the selection of antibodies and/or antibody fragments binding to HIV-1 *In vitro*, characterized in that at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25 is used to bind to said antibodies and/or antibody fragments, whereafter the resulting molecules are separated and purified according to standard techniques.
12. A method for the determination of the status of infection of HIV-1 infected humans and/or animals *in vitro*, characterized in that at least one of the amino acid sequences of the SEQ ID NO: 1 through SEQ ID NO: 25 is added to a serum of an infected patient and/or experimental animal, whereupon an HIV-1 neutralization titer is determined following state of the art methods.
13. Use of peptides or fusion proteins manufactured according to anyone of Claims 1 to 10 to select antibodies and/or antibody/fragments binding to HIV-1 *In vitro* and/or to determine neutralization titers in sera of humans or animals, or peptide/fragments binding to HIV-1.
14. Use of peptides or fusion proteins manufactured according to anyone of Claims 1 to 10 for the manufacture of a vaccine to block HIV-1 neutralizing antibodies in humans.
15. Use according to claim 14 to prevent progression of HIV-1 infection to AIDS
16. Use of peptides or fusion proteins manufactured according to anyone of claims 1 to 10 for the manufacture of antidiotypic antibodies.
17. Peptides binding to antibodies that show neutralizing activity against different strains and clinical isolates of HIV-1 and that inhibit the fusion of cells caused by HIV-1, characterized in that said peptides are composed according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25.
18. Peptides according to claim 17, characterized in that they are genetically encoded by the nucleotide sequence according to anyone of the SEQ ID NO: 28 through SEQ ID NO: 50 or by sequences that are deduced from SEQ ID NO: 28 through SEQ ID NO: 50 by degeneration.
19. Peptides according to claim 17 or 18, which upon injection into a mammal either alone or in combination with an adjuvant cause an immune response that leads to the generation of HIV-1 neutralizing antibodies.
2. Peptide nach Anspruch 1, dadurch gekennzeichnet, daß sie genetisch durch die Nukleotidssequenz gemäß einer
20. Peptides according to anyone of claims 17 to 19 in combination with an adjuvant, wherein the adjuvant is a substance to which said peptides are bound through chemical interaction.
21. Peptides according to claim 20 in the form of fusion peptides, characterized in that a protein or part of a protein is used as the adjuvant, to which said peptides are bound by fusion of the respective nucleotide sequences and subsequent expression of the fusion genes in a biological expression system.
22. Fusion peptides according to claim 21, characterized in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 are used as linker or as part thereof in order to link the variable domains of a single chain Fv fragment.
23. Fusion peptides according to claim 21, characterized in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a monoclonal antibody.
24. Fusion peptides according to SEQ ID NO: 1 through SEQ ID NO: 25 are expressed as part of one or more hypervariable regions of a monoclonal antibody.
25. Fusion peptides according to anyone of claims 21, 23 or 24, characterized in that they are either expressed, or chemically or enzymatically synthesized as part of a single chain Fv fragment or as part of a Fab fragment.
26. Fusion peptides according to claim 21, characterized in that one or more peptides according to anyone of the SEQ ID NO: 1 through SEQ ID NO: 25 substitute one or more parts of the peptide sequence of a viral protein, or are inserted into antigenic sites of a viral protein.
27. Fusion peptides according to claim 26, characterized in that they are part of a virus.
28. Fusion peptides according to claim 26 or 27, characterized in that the viral protein is the hemagglutinin or neuraminidase of influenza virus.
29. Fusion peptides according to claim 26 or 27, characterized in that the viral protein is the surface antigen or the core antigen of hepatitis B virus.
30. Use of peptides as defined in anyone of claims 17 through 21 to select antibodies and/or antibody/fragments binding to HIV-1 *In vitro*.
31. Use of peptides as defined in anyone of claims 17 through 21 in an immunological test *in vitro* to determine the neutralization titer in complete sera of patients or experimental animals infected with HIV-1, or to determine the status of infection or to make a prognosis on the further progress of infection.
32. Use of peptides as defined in anyone of claims 17 to 21 for the manufacture of antidiotypic antibodies.
33. A vaccine against HIV-1, characterized in that it comprises at least one peptide and/or fusion peptide as defined in anyone of claims 17 to 29 and/or at least one antidiotypic antibody obtained according to claim 16.
- Patentansprüche
- 50
- Patentansprüche für folgende Vertragsstaaten: BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE
1. Peptide, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß die Peptid gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 zusammengesetzt sind.
- 55

der SEQ ID NO: 26 bis SEQ ID NO: 50 kodiert ist oder durch Sequenzen, welche durch Degenerierung aus SEQ ID NO: 26 bis SEQ ID NO: 50 abgeleitet sind.

Patentsprüche für folgenden Vertragsstaat: ESS

3. Peptide nach Anspruch 1 oder 2, welche bei Infektion in ein Säugertier, entweder alleine oder in Kombination mit einem Adjunktiv, eine Immunreaktion verursachen, welche zur Erzeugung von HIV-1 neutralisierenden Antikörpern führt.

4. Peptido nach einem der Ansprüche 1 bis 3, in Kombination mit einem Adjunktiv, wobei das Adjunktiv eine Substanz ist, an welche die Peptide durch chemische Wechselwirkung gebunden sind.

5. Peptido nach Anspruch 4, in Form von Fusionopeptiden, dadurch gekennzeichnet, daß ein Protein oder ein Teil eines Proteins als Adjunktiv benutzt wird, an welches die Peptide durch Fusion der jeweiligen Aminosäuresequenzen und nachfolgende Expression der Fusionsgene in einem biologischen Expressionssystem gebunden sind.

6. Fusionopeptide nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Bindungsobjekt oder als dessen Teil benutzt werden, um die variablen Domänen eines Fv-Fragmentes mit einziger Kette zu verbinden.

7. Fusionopeptid nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidketenz eines monoklonalen Antikörpers substituieren.

8. Fusionopeptide nach den Ansprüchen 5 und 7, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Teil einer oder mehrerer hypervariabler Regionen eines monoklonalen Antikörpers ausgedehnt sind.

9. Fusionopeptide nach einem der Ansprüche 7, 7 oder 8, dadurch gekennzeichnet, daß sie entweder als Teil eines Fv-Fragments mit einziger Kette oder als Teil eines Fab-Fragments ausgedehnt oder chemisch oder enzymatisch synthetisiert sind.

10. Fusionopeptide nach Anspruch 5, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 Jahren oder mehrere Teile der Peptidketenz eines viralen Proteins substituierten oder an angewisse Stellen eines viralen Proteins eingesetzt sind.

11. Fusionopeptide nach Anspruch 10, dadurch gekennzeichnet, daß sie Teil eines Virus sind.

12. Fusionopeptide nach den Ansprüchen 10 oder 11, dadurch gekennzeichnet, daß das virale Protein das Hämagglutinin oder die Neuraminidase eines Grippovirus ist.

13. Fusionopeptide nach den Ansprüchen 10 oder 11, dadurch gekennzeichnet, daß das virale Protein das Oberflächenantigen oder das Kernantigen eines Hepatitis-B-Virus ist.

14. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, zum Selektieren von mit HIV-1 *in vitro* eine Bindung eingehenden Antikörpern oder Antikörperfragmenten.

15. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, in einem immunologischen Test *in vitro*, um den Neutralisationsindex in den vollständigen Serum von Patienten oder von mit HIV-1 infizierten Vorsuchstieren zu bestimmen oder den Status einer Infektion zu bestimmen oder eine Prognose über den weiteren Fortschritt einer Infektion zu stellen.

16. Verwendung von Peptiden nach einem der Ansprüche 1 bis 5, zur Herstellung von antiviralen Antikörpern.

17. Vaccine gegen HIV-1, dadurch gekennzeichnet, daß es weniger ein Peptid und oder Fusionopeptid nach einem der Ansprüche 1 bis 13 und oder mindestens einen nach Anspruch 16 erhaltenen antiviralen Antikörpern aufweist.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die Peptide als Fusionopeptide mit Glutathion-S-Transferase (GST) ausgedrückt werden, wobei die Oligonukleotids vorzugsweise hybridisiert und zwischen den Bam HI - und der Eco RI-Stelle des Plasmids pGEX-2T (Pharmacia) gelöst werden.

3. Verfahren nach Anspruch 1 oder 2, dadurch gekennzeichnet, daß die E. coli-Zellen nach dem Ausdrucken der Aminosäuresequenzen und/oder Peptide aufgetrocknet und die Aminosäuresequenzen und/oder Peptide von der flüssigen Fraktion abgetrennt und gereinigt werden.

4. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß das Aufbrechen der E. coli-Zellen durch Beschaffung erreicht wird.

5. Verfahren nach Anspruch 3 oder 4, dadurch gekennzeichnet, daß des Abtrennen und die Reinigung der Aminosäuresequenzen und/oder Peptide durch Affinitätschromatographie, vorzugsweise unter Verwendung einer Glutathion-S-Transferase-4B-Komplexe, durchgeführt wird, wobei die Aminosäuresequenzen und/oder Peptide, vorzugsweise mit einer Glutathion, NaCl und einem Puffer enthaltenden Lösung elutieren werden.

6. Verfahren nach Anspruch 5, dadurch gekennzeichnet, daß das Eluterungsmaterial eine aus 20 mM Glutathion und 120 mM NaCl in 10 mM Tris-HCl zusammengesetzte Lösung, pH-Wert 8,0, ist.

7. Verfahren zur Herstellung von Fusionopeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß

a) wenigstens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 durch Malagassen *In vitro* an Antigenstämme des Hämagglutinin des Grippe-A-Virus eingesetzt wird und dadurch zu schimmärischen DNA-Konstruktionen führt,

b) wonach die schimmärischen DNA-Konstruktionen in ein Grippe-HKWSN-Virus RNP-konstituiert werden, wodurch schimmärische Grippe/HV-Viren geschaffen werden, die antigene Eigenschaften des Fusionopeptids aufweisen, wobei die schimmärische Grippe/HV-Viren vorzugsweise zum Induzieren einer neutralisierenden Immunreaktion gegen genetisch divergierende HIV-1-Stämme fähig sind.

8. Verfahren zur Herstellung von Fusionopeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß

a) es wird eine Fv-Konstruktion eines neutralisierenden Anti-HIV-gp 120-Antikörpers mit einer einzigen Kette hergestellt,

b) wenigstens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 wird in das Bindungsobjekt eingebracht, die die variablen Regionen des schwäben und der leichten Kette eines Immunoglobulin-Moleküls verbindet,

c) die Fusionopepteine werden sodann als Teil des eingesetzten Aminosäuresequenz enthaltenden Immunoglobulin-Moleküls ausgedrückt.

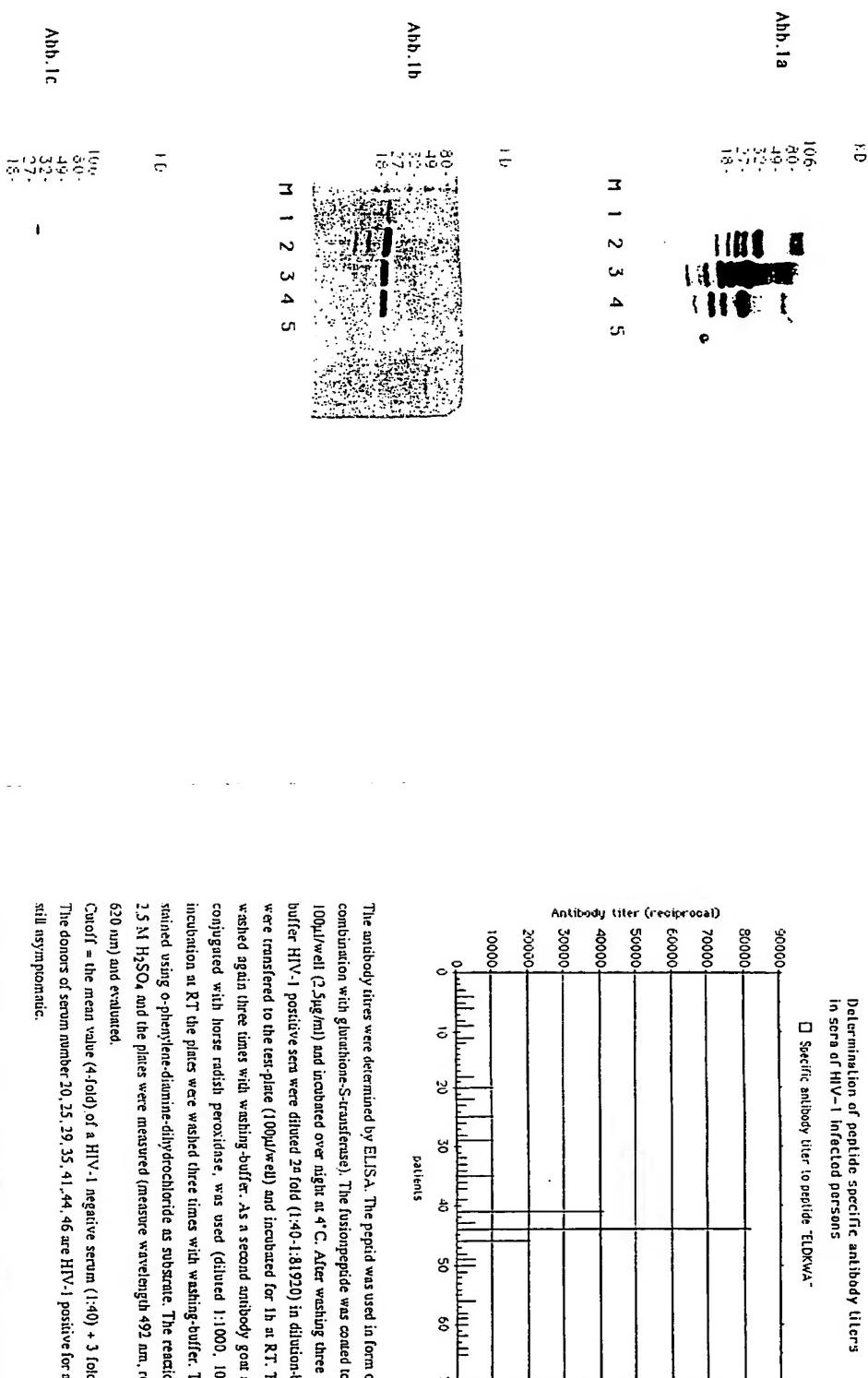
9. Verfahren zur Herstellung von Fusionopeptiden, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß ein oder mehrere hypervariablen(Bereiche) oder Teile davon eines monoklonalen Antikörpers durch mindestens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 unter Anwendung von Sämtkrohverfahren substituiert wird (wirden).

10. Verfahren nach Anspruch 9, dadurch gekennzeichnet, daß die Fusionospptide in *E. coli* mit einziger Kette ausgedrückt werden, dann abgeschnitten und in Mäuse injiziert werden, was dabei zur Bildung von antikörperplastischen Antikörpern führt, die dazu fähig sind, eine neutralisierende Immunreaktion gegen genetisch abweigende HIV-1-Stämme zu induzieren.
11. Verfahren zur Auswahl von Antikörpern und/oder Antikörpern und/oder Antikörpern, die dazu fähig sind, eine neutralisierende Immunreaktion gegen genetisch abweigende HIV-1-Stämme zu induzieren.
12. Verfahren zum Bestimmen des Infektionsstatus von mit HIV-1 infizierten Menschen und/oder Tieren *in vitro*, dadurch gekennzeichnet, daß mindestens eine der Aminosäuresequenzen SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Peptidketten abgliedert, wodurch ein HIV-1-Neutralisationsstifter nach Serum eines infizierten Patienten und/oder Versuchstiere zugeliefert wird, worauf die sich ergebenden Moleküle nach Standardverfahren abgetrennt und gereinigt werden.
13. Verwendung von Peptiden oder Fusionospptide, die nach einem der Ansprüche 1 bis 10 hergestellt sind, um Antikörper und/oder Antikörper-Fragmente auszuwählen, die mit HIV-1 *in vitro* eine Bindung eingehen und/oder zum Bestimmen von Neutralisationsstiftern in Sera von Menschen oder Tieren.
14. Verwendung von Peptiden oder Fusionospptide, die nach einem der Ansprüche 1 bis 10 hergestellt sind, für die Herstellung eines Vaccins zum Hervorrufen von HIV-1 neutralisierenden Antikörpern bei Menschen.
15. Verwendung nach Anspruch 14, zum Verhindern des Fortschreitens einer HIV-1-Infektion zu AIDS.
16. Verwendung von Peptiden oder Fusionospptide, die nach einem der Ansprüche 1 bis 10 hergestellt sind, für die Erzeugung von antikörperplastischen Antikörpern.
17. Peptide, welche mit Antikörpern eine Bindung eingehen, die eine neutralisierende Aktivität gegenüber verschiedenen Stämmen und klinischen Isolaten von HIV-1 zeigen, und welche die durch HIV-1 verursachte Zellfusion hemmen, dadurch gekennzeichnet, daß die Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 zusammenge setzt sind.
18. Peptid nach Anspruch 17, dadurch gekennzeichnet, daß sie genetisch durch die Nukleotidsequenz, gemäß einer der SEQ ID NO: 25 bis SEQ ID NO: 50 kodiert ist oder durch Sequenzen, welche durch Degeneration aus SEQ ID NO: 25 bis SEQ ID NO: 50 abgeleitet sind.
19. Peptide nach Anspruch 17 oder 18, welche bei Infektion in ein Säugeler, entweder alleine oder in Kombination mit einem Adjuvans, eine Immunreaktion verursachen, welche zur Erzeugung von HIV-1 neutralisierenden Antikörpern führt.
20. Peptide nach Anspruch 17 oder 18, welche bei Infektion in ein Säugeler, entweder alleine oder in Kombination mit einem Adjuvans, eine Immunreaktion verursachen, welche zur Erzeugung von HIV-1, charakterisiert anhand der Sequenzen 1 bis 19, in Kombination mit einem Adjuvans, wobei das Adjuvans eine Substanz ist, an welche die Peptide durch chemische Wechselwirkung gebunden sind.
21. Peptide nach Anspruch 20, in Form von Fusionospptide, dadurch gekennzeichnet, daß ein Protein oder ein Teil eines Proteins als Adjuvans benutzt wird, an welches die Peptide durch Fusion der jeweiligen Nukleotidsequenzen und nachfolgende Expression der Fusionospide in einem biologischen Expressionssystem gebunden sind.
22. Fusionospide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Bindungsstiel oder als dessen Teil benutzt werden, um die variablen Domänen eines Fv-Fragments mit einziger Kette zu verbinden.
23. Fusionospide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehrere Teile der Peptidsequenz eines monoklonalen Antikörpers substituieren.
24. Fusionospide nach den Ansprüchen 21 und 23, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 als Teil einer oder mehrerer hypervariabler Regionen eines monoklonalen Antikörpers ausgedrückt sind.
5. Fusionospide nach Anspruch 21, dadurch gekennzeichnet, daß ein oder mehrere Peptide gemäß einer der SEQ ID NO: 1 bis SEQ ID NO: 25 einen oder mehreren Teils der Peptidsequenz eines viralen Proteins substituiert oder in anliegende Stellen eines viralen Proteins eingesetzt sind.
6. Fusionospide nach Anspruch 26, dadurch gekennzeichnet, daß sie Teil eines Virus sind.
10. Fusionospide nach den Ansprüchen 26 oder 27, dadurch gekennzeichnet, daß das virale Protein das Hämagglutinin oder die Neuraminidase eines Grippevirus ist.
15. Fusionospide nach den Ansprüchen 26 oder 27, dadurch gekennzeichnet, daß das virale Protein das Oberflächenantigen oder das Kompetitor eines Hepatitis-B-Virus ist.
20. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zum Selektieren von mit HIV-1 *in vitro* eine Bindung eingehenden Antikörpern oder Antikörperfragmenten.
25. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zur Herstellung von antikörperplastischen Antikörpern.
30. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zum Selektieren von mit HIV-1 *in vitro* eine Bindung eingehenden Antikörpern oder Antikörperfragmenten.
35. Verwendung von Peptiden nach einem der Ansprüche 17 bis 21, zur Herstellung von antikörperplastischen Antikörpern.
40. Revendications pour les Etats contractants suivants : BE, CH, DE, DK, FR, GB, IE, IT, LI, NL, PT, SE
1. Peptides se lient à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et/ou isolats cliniques du HIV-1 et qui inhibent la fusion des cellules induite par le HIV-1, caractérisés en ce qu'en les peptides sont composés selon une quelconque des Séquences identifiées par les numéros 1 à 25.
 2. Peptides selon la revendication 1, caractérisés en ce qu'ils sont généralement produits par la séquence nucléotidique selon l'une quelconque des Séquences identifiées par les numéros 26 à 50 ou les séquences qui sont déduites des Séquences identifiées par les numéros 26 à 50 par dégénérescences.
 3. Peptides selon la revendication 1 ou 2, qui suite à leur injection à un mammifère, soit séparément soit en composition avec un adjuvant, induisent une réponse immunitaire qui conduit à la production d'anticorps neutralisants anti-HIV.
 4. Peptides selon la revendication 1 ou 2, qui suite à leur injection à un mammifère, soit séparément soit en composition avec un adjuvant, induisent une réponse immunitaire qui conduit à la production d'anticorps neutralisants anti-HIV.
 5. Peptides selon l'une quelconque des revendications 1 à 3 en association avec un adjuvant, dans lesquels l'adjuvant est une substance à laquelle lesdits peptides se trouvent liés par liaison chimique.
 6. Peptides selon l'une quelconque des revendications 1 à 3 en association avec un adjuvant, dans lesquels l'adjuvant est une substance à laquelle lesdits peptides se trouvent liés par liaison chimique.
55. Peptides selon la revendication 4 sous forme de peptides de fusion, caractérisés en ce qu'une protéine ou une partie d'une protéine est utilisée comme adjuvant, à laquelle lesdits peptides sont liés par liaison des séquences nucleotidiques et expression ultérieure des gènes de fusion dans un système d'expression biologique.
60. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont utilisés comme segment de jonction ou comme une partie de l'oligonucléotide dans de jondre les domaines variables d'un fragment Fv à chaîne unique.

7. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptide d'un anticorp monoclonal.
- 5 8. Peptides de fusion selon les revendications 5 ou 7, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 sont exprimés sous forme d'une partie d'une ou de plusieurs régions hypervariables d'un anticorp monoclonal.
9. Peptides de fusion selon l'une quelconque des revendications 7 ou 8, caractérisés en ce qu'ils sont soit exprimés, soit synthétisés par voie chimique ou enzymatique sous forme d'un peptide d'un fragment Fv à chaîne unique ou d'un peptide d'un fragment Fab.
10. Peptides de fusion selon la revendication 5, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des Séquences identifiées par les numéros 1 à 25 soient substitués à une ou plusieurs parties de la séquence peptide d'un protéine virale, ou sont insérés dans des sites antigéniques d'une protéine virale.
- 15 11. Peptides de fusion selon la revendication 10, caractérisés en ce qu'ils sont issus d'un virus.
- 20 12. Peptides de fusion selon la revendication 10 ou 11, caractérisés en ce que la protéine virale est l'hémagglutinine ou la neuramidase du virus de la grippe A.
- 25 13. Peptides de fusion selon la revendication 10 ou 11, caractérisés en ce que la protéine virale est l'hémagglutinine de surface ou l'antigène nuclocapside du virus de l'hépatite B.
- 30 14. Utilisation de peptides lors qui définis dans l'une quelconque des revendications 1 à 5 pour sélectionner des anticorps ou des fragments d'anticorps se liant au HIV-1 *in vitro*.
15. Utilisation de peptides tels que définis dans l'une quelconque des revendications 1 à 5 dans un essai immunologique *in vitro* pour déterminer le titre d'anticorps neutralisants sur sérum total de patients ou d'animaux de laboratoire infectés par le HIV-1, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.
- 35 16. Utilisation de peptides tels que définis dans l'une quelconque des revendications 1 à 5 pour l'obtention d'anticorps anti-idiotypiques.
17. Vaccin contre le HIV-1, caractérisé en ce qu'il comprend au moins un peptide et/ou peptide de fusion tel que défini dans l'une quelconque des revendications 1 à 13 et/ou au moins un anticorp anti-idiotype obtenu selon la revendication 16.
- 40 Revendications pour l'Etat contractant suivant : ES
1. Procédé de production de peptides se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de souches à d'isols cliniques génétiquement divergents du HIV-1, et qui inhibent la fusion de cellules indiquée par le HIV-1, caractérisé en ce que les oligonucléotides correspondants à une des séquences d'acides aminés encodées par les séquences identifiées par les numéros 1 à 25 sont clonés, transformés et exprimés dans E. coli de préférence dans E. coli DH5 α .
- 45 2. Procédé selon la revendication 1, caractérisé en ce que les peptides sont exprimés comme protéines de fusion à la glutathion-S-transférase (GST), de préférence en hybridant et en clonant des oligonucléotides entre le site Bam-HI et Eco RI du plasmide pGEX-2T (Pharmacia).
- 50 3. Procédé selon la revendication 1 ou 2, caractérisé en ce que suit à l'expression descriptes séquences d'acides aminés et/ou des peptides, il y a rupture des cellules E. coli et séparation et purification des séquences d'acides aminés et/ou des peptides de la fraction liquide.
- 55 4. Procédé selon la revendication 3, caractérisé en ce que la rupture des cellules E. coli s'obtient par sonication.
5. Procédé selon la revendication 3 ou 4, caractérisé en ce que la séparation et la purification des séquences d'acides aminés et/ou des peptides se effectuent par chromatographie distincte à l'aide d'une colonne de type glutathione sépharose 4B, à partir de laquelle les séquences d'acides aminés abus les peptides sont elués, de préférence avec une solution contenant du glutathione, NaCl et un tampon.
6. Procédé selon la revendication 5, caractérisé en ce qu'environnement de dissolution est une solution composée de glutathione 20 mM et de NaCl 120 mM dans du Tris 100 mM-HCl, pH 8,0.
7. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce que
- a) au moins l'une des séquences d'acides aminés correspondant aux séquences identifiées par les numéros 1 à 25 est introduite dans des sites antigéniques de l'hémagglutinine du virus de la grippe A par mutagénèse *in vitro*, de manière à obtenir des constructions d'DNA chimères,
 - b) lesdites constructions d'DNA chimères sont ensuite transférées par RNP dans des virus de la grippe HK/WSN,
 - c) au moins l'une des séquences d'acides aminés correspondant aux séquences identifiées par les numéros 1 à 25 est introduite dans le segment de jonction qui relie les régions variables de la chaîne lourde et légère d'un molecule d'immunoglobuline,
 - d) lesdites protéines qui présentent une activité neutralisante vis-à-vis de souches de HIV-1 généralement l'hémagglutinateur.
8. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce que
- a) une construction Fv à chaîne unique d'un anticorp anti-gp120 de HIV est préparée,
 - b) au moins l'une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 est insérée dans le segment de jonction qui relie les régions variables de la chaîne lourde et légère d'un molecule d'immunoglobuline,
 - c) lesdites protéines de fusion sont ensuite exprimées sous forme d'une partie de laclie molécule d'immunoglobuline contenant la séquence d'acides aminés insérée.
9. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce qu'il existe plusieurs régions hypervariables qui partagent des parties de telles régions d'un anticorp monoclonal.
- 35 10. Procédé d'obtention de protéines de fusion se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisé en ce qu'il existe plusieurs régions hypervariables qui partagent des parties de telles régions d'un anticorp monoclonal.
- 30 11. Procédé de sélection d'anticorps et/ou de fragments d'anticorps se liant au HIV-1 *in vitro*, caractérisé en ce qu'il existe au moins une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 et utilisée pour fixer les isolats et/ou fragments d'anticorps d'anticorps.
- 35 12. Procédé de détermination *in vitro* du stade d'infection chez des animaux infectés par le HIV-1, caractérisé en ce qu'il existe une des séquences d'acides aminés correspondant aux Séquences identifiées par les numéros 1 à 25 est ajoutée à un sérum d'un malade infecté et/ou d'un animal de laboratoire, à la dilution par les numéros 1 à 25 est ajoutée à un sérum d'un malade infecté et/ou d'un animal de laboratoire, à la dilution de quoi le titre d'anticorps neutralisants anti-HIV-1 est déterminé suivant des techniques conventionnelles.
- 40 13. Utilisation de peptides et/ou protéines de fusion obtenus selon l'une quelconque des revendications 1 à 10 pour sélectiver des anticorps et/ou des fragments d'anticorps se liant au HIV-1 *in vitro* et/ou pour déterminer les titres d'anticorps neutralisants dans les sérum humains ou animaux.

14. Utilisation de peptides ou de protéines de fusion obtenus selon l'une quelconque des revendications 1 à 10 dans l'obtention d'un vaccin destiné à induire la production d'anticorps neutralisants anti-HIV-1.
15. Utilisation selon la revendication 14 pour empêcher l'évolution de l'infection par le HIV-1 vers le SIDA.
16. Utilisation de peptides ou de protéines de fusion obtenues selon l'une quelconque des revendications 1 à 10 pour l'obtention d'anticorps anti-HIV-1.
17. Peptides se liant à des anticorps qui présentent une activité neutralisante vis-à-vis de différentes souches et isolats cliniques du HIV-1 et qui inhibent la fusion de cellules induite par le HIV-1, caractérisés en ce que lesdits peptides sont composés selon l'une quelconque des séquences identifiées par les numéros 1 à 25.
18. Peptides selon la revendication 17, caractérisés en ce qu'ils sont générés/mutés/encodés par la séquence nucléotidique selon l'une quelconque des séquences identifiées par les numéros 26 à 50 ou des séquences qui sont dérivées des séquences identifiées par les numéros 1 à 25.
19. Peptides selon la revendication 17 ou 18, qui suite à leur injection dans un mammifère, soit séparément soit en conjonction avec un adjuvant, induisent une réponse immune qui conduit à la production d'anticorps neutralisants anti-HIV-1.
20. Peptides selon l'une quelconque des revendications 17 à 19 en association avec un adjuvant, dans lesquels l'adjuvant est une substance à laquelle lesdits peptides se trouvent liés par liaison chimique.
21. Peptides selon la revendication 20 sous forme de peptides de fusion, caractérisés en ce qu'un protéine ou une partie de protéine est utilisée comme adjuvant, à laquelle cesdits peptides sont liés par fusion des séquences nucléotidiques respectives et expression ultérieure des gènes de la fusion dans un système d'expression biologique.
22. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des séquences identifiées par les numéros 1 à 25 sont utilisés comme protéine ou comme une partie de tel segment afin de joindre les domaines variables d'un fragment Fv à chaîne unique.
23. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptides d'un anticorp monoclinal.
24. Peptides de fusion selon les revendications 21 et 23 caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des séquences identifiées par les numéros 1 à 25 sont exprimés sous forme d'une partie d'une ou de plusieurs régions hypervariables d'un anticorp monoclinal.
25. Peptides de fusion selon l'une quelconque des revendications 21, 23 ou 24, caractérisés en ce qu'ils sont soit exprimés, soit synthétisés par voie chimique ou enzymatique sous forme d'une partie d'un fragment Fv à chaîne unique ou d'une partie d'un fragment Fab.
26. Peptides de fusion selon la revendication 21, caractérisés en ce qu'un ou plusieurs peptides selon l'une quelconque des séquences identifiées par les numéros 1 à 25 se substituent à une ou plusieurs parties de la séquence peptides d'une protéine virale, ou sont insérées dans des sites antigéniques d'une protéine virale.
27. Peptides de fusion selon la revendication 26, caractérisés en ce qu'ils sont partie d'un virus.
28. Peptides de fusion selon la revendication 26 ou 27, caractérisés en ce que la protéine virale est l'hémagglutinine ou la neuramidase du virus de la grippe.
29. Peptides de fusion selon la revendication 26 ou 27, caractérisés en ce que la protéine virale est l'antigène de surface ou l'antigène nuclocapillaire du virus de l'hépatite B.
30. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 pour sélectionner des anticorps ou des fragments d'anticorps se liant au HIV-1 *in vitro*.

31. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 dans un essai immuno-logique *in vitro* pour déterminer le taux d'anticorps neutralisants sur serum total de patients ou d'animaux de laboratoire infectés par le HIV-1, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.
32. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 pour l'obtention d'anticorps anti-HIV-1.
33. Vaccin contre le HIV-1, caractérisé en ce qu'il comprend au moins un peptide et/ou peptide de fusion tel que défini dans l'une quelconque des revendications 17 à 29 et/ou au moins un anticorp anti-HIV-1 obtenu selon la revendication 16.
34. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 dans un essai immuno-logique *in vitro* pour déterminer le taux d'anticorps neutralisants sur serum total de patients ou d'animaux de laboratoire infectés par le HIV-1, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.
35. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 dans un essai immuno-logique *in vitro* pour déterminer le taux d'anticorps neutralisants sur serum total de patients ou d'animaux de laboratoire infectés par le HIV-1, ou pour déterminer le stade d'infection ou pour formuler un pronostic sur l'évolution de l'infection.
36. Utilisation de peptides tels que définis dans l'une quelconque des revendications 17 à 21 pour l'obtention d'anticorps anti-HIV-1.
37. Vaccin contre le HIV-1, caractérisé en ce qu'il comprend au moins un peptide et/ou peptide de fusion tel que défini dans l'une quelconque des revendications 17 à 29 et/ou au moins un anticorp anti-HIV-1 obtenu selon la revendication 16.



The antibody titres were determined by ELISA. The peptide was used in form of a fusionpeptide (in combination with glutathione-S-transferase). The fusionpeptide was coated to 96 microtitre plates (100 μ l/well (2.5 μ g/ml)) and incubated over night at 4°C. After washing three times with washing-buffer, HIV-1 positive sera were diluted 2ⁿ fold (1:40-1:81920) in dilution-buffer and aliquotes were transferred to the test-plate (100 μ l/well) and incubated for 1 h at RT. Then the plates were washed again three times with washing-buffer. As a second antibody goat anti human γ -chain, conjugated with horse radish peroxidase, was used (diluted 1:1000, 100 μ l/well). After 1 h incubation at RT the plates were washed three times with washing-buffer. Then the plates were stained using α -phenylene-diamine-diimidochloride as substrate. The reaction was stopped with 2.5 M H₂SO₄ and the plates were measured (measure wavelength 492 nm, reference wavelength 620 nm) and evaluated.

Cutoff = the mean value (4-fold) of a HIV-1 negative serum (1:40) + 3 fold standard deviation. The donors of serum number 20, 25, 29, 35, 41, 44, 46 are HIV-1 positive for at least five years and still asymptomatic.

Figure 2: Graphic of the specific antibody titers to the peptide with the aminoacid sequence "ELDKWAA" of 63 sera from HIV-1 positive donors

Influenza/HIV inhibition of HIV-1 IIIB neutralization

Immunogen: Antibody titer:

K1	<10
K2	<10
K3	<10
F1	1600
F2	6400
F3	800
S1M	3200
S2M	400
S3M	800
V1	3200
V2	3200
V3	6400

immunological antibodies: residual IIIB neutralizations titer in % after incubation with:

Mock	Influenza WSN	Influenza/HIV
100%	100%	10%
100%	100%	10%
100%	100%	10%

Figure 3a: Influenza/HIV inhibition of HIV-1 neutralization. Results are expressed as reciprocal of the serum dilution giving > 90% reduction in HIV titer following preincubation of the mAbs 2F5 and 2G12 with culture medium (Mock), influenza WSN or influenza/HIV. 2F5 is the monoclonal antibody specific for the different epitope on gp160. Residual HIV-neutralizing activity was determined by incubating dilutions of the antibody/virus mixture with 10^3 infectious units (TCID₅₀) of HIV-1 IIIB for 1 h at 37°C. Aliquots (100µl) of medium containing 10^3 C8166 cells were added and the presence of syncytia recorded after 48 h as an indication of HIV infection.

Figure 3b: Antibody titer. Three Balb/c mice each were immunized with either 100µg CST (K), 10µg Fusion protein (F), 10µg of the "immunological supermolecule" (100µg of the recombinant antiidiotype antibody (rA) or 4.0 log₁₀TCID₅₀ of the recombinant influenza/HIV virus (V) and were boosted after 2 and 4 weeks. One week after the last immunization ELISA antibody were determined. Results are given as reciprocal values that gave significant positive values. The cutoff was the double value of a normal mouse serum. Recombinant gp160 was used as an antigen.

Reciprocal neutralization titers of HIV-1 isolates

Antisera	IIIB	RF	MN
P1	40	40	40
P2	80	40	40
P3	40	20	20
V1	40	80	40
V2	20	40	<10
V3	160	80	80
SM1	20	40	40
SM2	40	80	80
SM3	<10	<10	<10
rA1	40	40	40
rA2	40	80	20
rA3	80	40	40

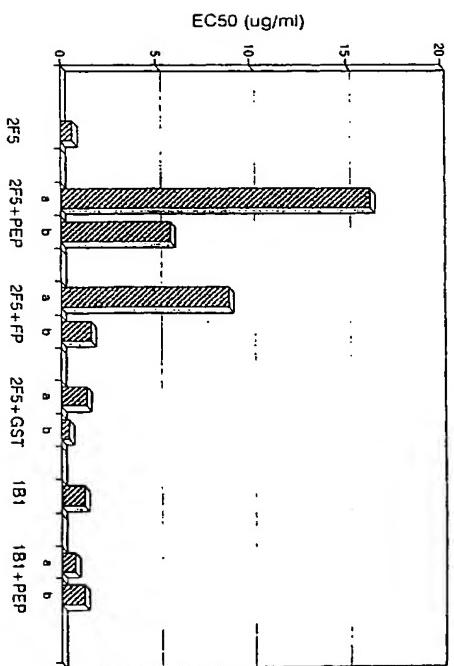


Fig. 4: Inhibition of neutralization by peptides. Synthetic peptide (PEP), fusion peptide (FP), and glutathione-S-transferase (GST) were preincubated with human 2F5 or 1B1 for 1 h at 37 °C, and then a syncytia inhibition assay was performed.

Antibodies were diluted in 2-fold steps starting with 5 µg/well.

PEP, synthetic peptide RLDKWA (peptide corresponding to SEQ ID NO: 1); a 25 µg, b 5 µg per well;

FP, fusion peptide RLDKWA with GST: a 25 µg, b 5 µg per well;

GST, glutathione-S-transferase: a 25 µg, b 5 µg per well;

1B1, neutralizing anti gp120 human supermolecule; rA ... recombinant antibody. Reciprocal neutralization titers of all controls were lower than 10.

Figure 3c: Neutralization of HIV-1 infection. Neutralization titers were determined by incubating 10µl of heat inactivated antiserum with 40µl virus supernatant containing 10³ infectious units of HIV-1 at 37°C for 1h. Residual HIV-1 infectivity was measured as described in fig. 3a.

Abbreviations: P ... fusion peptide. V ... chimeric influenza/HIV virus. SM ... "immunological supermolecule". rA ... recombinant antibody. Reciprocal neutralization titers of all controls were lower than 10.

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